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Thesis

THE ENDOCRINE SYSTEM OF THE GOLDEN HAMSTER (*CRICETUS AURATUS WATERHOUSE*)

BY

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INTRODUCTION

The Syrian or golden hamster (Cricetus auratus Waterhouse) is being used more and more extensively in the laboratory. Several attributes of the animal make it well suited for experimental work. For example, the hamster has the shortest gestation period of any mammal known, sixteen days; it can breed frequently; the litters are large, from eight to eighteen; and the hamster is relatively clean as compared to the rat and other rodents. All of these qualities combined provide an animal that is eminently suitable for various fields of physiological investigation.

A survey of the literature discloses that with the exception of the reproductive system, little or no work has been done on the morphology or the physiology of the endocrine system of the golden hamster. Many papers have been published on the importance of the European hamster (Cricetus cricetus L.) in relation to agriculture, but these are not reviewed in this thesis, since I consider them outside the present topic.

I shall present a survey of the available literature dealing with the estrous cycle of the hamster, the action of sex hormones on the estrous cycle, the hormonal modification of sex development by various sex and gonadotrophic hormones, the cyclic seasonal activity of the endocrine glands, and the relation of sex steroids to the adrenals. The chief difficulty involved in the preparation of this paper was the lack of con-

firmation, in the literature and the scarcity of publications upon any of the above mentioned topics. Therefore, I shall present the work done for what value it may have, and attempt to evaluate as much of it as I can.

As can be surmised from the foregoing paragraph, much remains to be done in the field of endocrinology of the hamster. To that end, I have conducted a series of bilateral adrenalectomies upon the hamster, and have presented the findings, observations, and conclusions derived therefrom in the body of this thesis.*

*I am indebted to Dr. Leland C. Wyman of the Boston University Biology Department for his invaluable advice and assistance in the developing of the operative techniques and in the conduct of the investigation.

CYCLIC SEASONAL ACTIVITY OF THE ENDOCRINE GLANDS

Kayser and Aron (1938) investigated the cyclic seasonal activity of the anterior pituitary, the thyroid, the adrenal cortex, and the male genitals of the European hamster, <u>Cricetus frumentaris</u>. Their observations are included in this paper because the golden hamster goes into pseudo-hibernation, beginning in October and ending in March.

Anterior Pituitary

In March, the anterior pituitary exhibits a very compact parenchyma, with the eosinophil and chromophil cells in very great majority as compared with the chromophobe elements. The eosinophil cells have a voluminous cytoplasmic body.

In September, the parenchyma is made up of cordons of tightly knit lobules of cells, interspersed with enlarged conjunctivo-vascular spaces. All the cells are much smaller than in March and contain a cytoplasmic body, which forms a shallow bed around the nucleus. The chromophobe type cells predominate. The groups of eosinophil cells are more spread out and scantier than in March, while the shape of their elements are in contrast to that which they had at that time.

Thyroid

The thyroid is extremely active in March, with the vesicles of the epithelium thickened and the colloid hollowed

by numerous and large vacuoles of absorption. In September, the thyroid displays little or no activity. The vesicles are thin, the colloid dense, and no vacuoles are present.

Adrenal Cortex

In March, the reticular zone of the adrenal cortex is composed of large cells, squeezed one against the other in many beds, where narrow capillaries circulate.

The histological picture is changed in September. Narrow rows of small cells border relatively large conjunctivo-vascular spaces.

Lipids were observed in March, but never in September. The animals displays a decline in activity between March and September, and the reverse between September and March. The evolution of the anterior pituitary precedes that of the thyroid and the testes.

Relation Of Temperature To Hibernation

It was found that the period of hibernation of the hamster may be lengthened or shortened by varying the environmental temperature. Increased temperatures cause the testes of animals, about to undergo hibernation, to show renewed spermatogenic activity. The eosinophil cells of the pituitary showed pronounced granulation upon the increase of environ-

mental temperature; the pancreas, thyroid, and adrenal remained involuted.

THE REPRODUCTIVE SYSTEM OF THE GOLDEN HAMSTER

Normal Development

Ortiz (1947) made the following observations upon the post-natal development of the reproductive system. In both sexes, the development of the entire system is slow during the first two weeks. There is a sudden acceleration of growth between the sixteenth and twenty-sixth days. In the female, the vagina opens when the body weight approximates eight grams (at about the tenth day). Follicular antra first appear at twenty-six days. At thirty days the first spontaneous estrus occurs. Corpora lutea are formed by the thirty-sixth day after birth.

In the male, beginning at sixteen days after birth, the tubules of the testes form lumina. At sixteen days, the ventral prostate acquires light areas, as the seminal vesicles do at twenty-six days. By twenty-six days, all the male accessory glands have reached their adult histological condition and there is evidence of secretion in the seminal vesicles and coagulating glands. The testes descend at about the twenty-sixth day, while sperm heads appear by the thirty-sixth day.

Hormonal Modification Of Reproductive System

The ovaries are first stimulated by gonadotrophin at ten days after birth. Hormone production and weight of the gland is increased. The reactivity of the ovary reaches its

peak at thirty-six days, characterized by great follicular stimulation and some thecal and interstitial luteinization, but no formation of corpora lutea (Ortiz, 1947). Peczenick (1942b) made the same observations, except that he found massive corpora lutea, in addition. The microscopic structure of the ovaries are not affected by androgens (Ortiz, 1947 and Bruner and Witschi, 1946). Estradiol benzoate was found to have no effect on ovarian weight (Ortiz).

The reactivity of the oviducts and uterus to gonadotrophins begins at ten days, decreasing after sixteen days (Ortiz). Bruner and Witschi found that androgens have no effect on the oviducts or uterus, but Ortiz found that testoterone propionate had an inhibitory effect up to sixteen days, with no effect after that. The latter investigator also found that estradiol benzoate exerted its greatest stimulating effect at six and ten days, with no effect after that day. Ortiz also observed that the vagina opened precociously at six days, after the injection of estradiol benzoate.

Gonadotrophins stimulated the growth of the testes at all ages (Ortiz, 1947). They also increased the production of sex hormones; the reactivity beginning at the tenth day, reaching its peak at the sixteenth day, and then decreasing until the thirty-sixth day. The interstitial tissue was slightly increased by gonadotrophins, at twenty-six and thirty-six days.

Testosterone propionate has a slight inhibitory action at younger ages, the peak of reactivity being at twenty-six days of age. Estradiol benzoate slightly reduces the weight of the testes and the production of the male hormone.

The ventral prostate, the seminal vesicles, and the coagulating glands are stimulated by gonadotrophins and androgens, the peak of reactivity being reached at sixteen days of age. These hormones also produce light areas in the seminal vesicles at sixteen days. Estradiol benzoate inhibits the growth of the ventral prostate, the seminal vesicles, and the coagulating gland at some ages.

Discussion

The work of Ortiz shows that the hamster does not continue the rapid prenatal growth in postnatal life, except in early maturation of the ovary, the establishment of estrous cycles, and the extremely precocious opening of the vagina. It also shows that the reproductive system of the hamster is less reactive to hormones than that of the rat.

The dependency of hormonal effect upon the receptivity of the end organ is especially brought out by Ortiz's report. It also points out many problems, which have not yet been answered. For example, why is the height of reactivity of the male organs reached at sixteen days? The fact that the ovaries and the female accessory organs displayed different ages, at which

they were most reactive to hormones, is also unexplained. Perhaps, the investigations described above can serve as a starting point for further investigation of the problem.

It seems that the investigation of Ortiz was carefully and accurately done, as the findings are confirmed by other workers, namely, Peczenick and Bruner and Witschi. This is one of the few instances that work on the golden hamster is confirmed by other investigators.

ESTROUS CYCLE OF THE GOLDEN HAMSTER

The estrous cycle of the golden hamster has been studied by many investigators, the first being Deanesly (1938), and the latest Ward (1946). Deanesly established the duration of the estrus cycle at four days, but stated that the stages can not be determined by vaginal smears. Yet, she said that a sticky opaque substance can be squeezed from the vagina every fourth morning, and called it the post-estrous stage. Later investigators agreed with the finding that the duration of the cycle is four days, but observed that the stages can be determined by vaginal smears.

There has been much disagreement among the workers in the field as to the terminology, description of the histological findings, and the assignment of the vaginal smears to their proper place in the cycle. In my opinion Ward (1946), has presented the most accurate description yet available. She has studied the cycle by external inspection, examination of vaginal smears, histological sections, and mating studies.

In aggreement with the terminology used for other animals, the day on which vulval discharge occurs is designated as day two and the day preceding it as day one. Ward's observations are presented in Table I.

Ward's description of proestrus agrees with that of Kent and Mixner (1945), Kent and Smith (1945), and of Kupperman, Greenblatt and Hair (1944), if their "cornified cells" are the

TABLE I

Stage	Time	Characterization
Stage 1 Proestrum	3 P.M. of day 1 to 6 P.M. of day 1.	Vaginal smears characterized by large numbers of non-nucleated epithelial cells, large and scale like in appearance, smaller number of nucleated epithelial cells and no leucocytes. (Fig. 1)
Stage 2 Estrus	6 P.M. of day 1 to 9 A.M. of day 2.	Vaginal smear characterized by the gradual appearance of many nucleated epithelial cells-columnar, elongated and oval. Many are vacuolated. A few large non-nucleated cells are present. (Fig. 2,3,4) Externally the last few hours of this stage are characterized by a sticky, white discharge from the vagina.
Stage 3 Metestrum A	9 A.M. of day 2 to noon of day 3.	Vaginal smear characterized by a gradual invasion of leucocytes, which reach their greatest numbers in the morning of day 3,and a gradual decrease in epithelial cells;oval epithelial cells are the predominant epithelial type present. The first hours of this stage may still by marked by the white vaginal discharge. (Fig. 5,6)
Stage 4 Metestrum* B	Noon of day 3 to afternoon (about 3 P.M.) of day 1.	Vaginal smears characterized by large amounts of amorphous debris, small munbers of epithelial cells (both nucleated and non-nucleated) and decreasing numbers of leucocytes. (Fig. 7)

*Ward uses the terminology advocated by Asdell (1946)in his book, "Patterns of Mammalian Reproduction". Asdel states,"... confine the term metestrum to the time during which estrogenic activity is declining and diestrum to the period in which the activity of the corpus luteum is paramount..". Metestrum B refers to that stage of the cycle called diestrum in previous descriptions of the estrous cycle of the hamster.

same as Ward's non-nucleated cells. Sheehan and Bruner (1945) are not in agreement at all, their description being few cornified cells for proestrus.

Ward's description of estrus is essentially the same as that of Peczenick (1942), but varies considerable from that of other investigators. Sheehan and Bruner (1945) observed that the cellular picture of estrus consists of a "few scattered small epithelial cells." According to Kent and Mixner (1945) and Kent and Smith (1945), the animal is in estrus when the field exhibits no leucocytes and squamous cells alone are present.

Peczenich (1942), Sheehan and Bruner (1945), and Ward, all agree upon the histological picture for metestrum. However, Kent and Mixner (1945) describe the metestrous smear as being characterized by the presence of elongated squamous cells, almost exclusively, and lasting for four to eight hours.

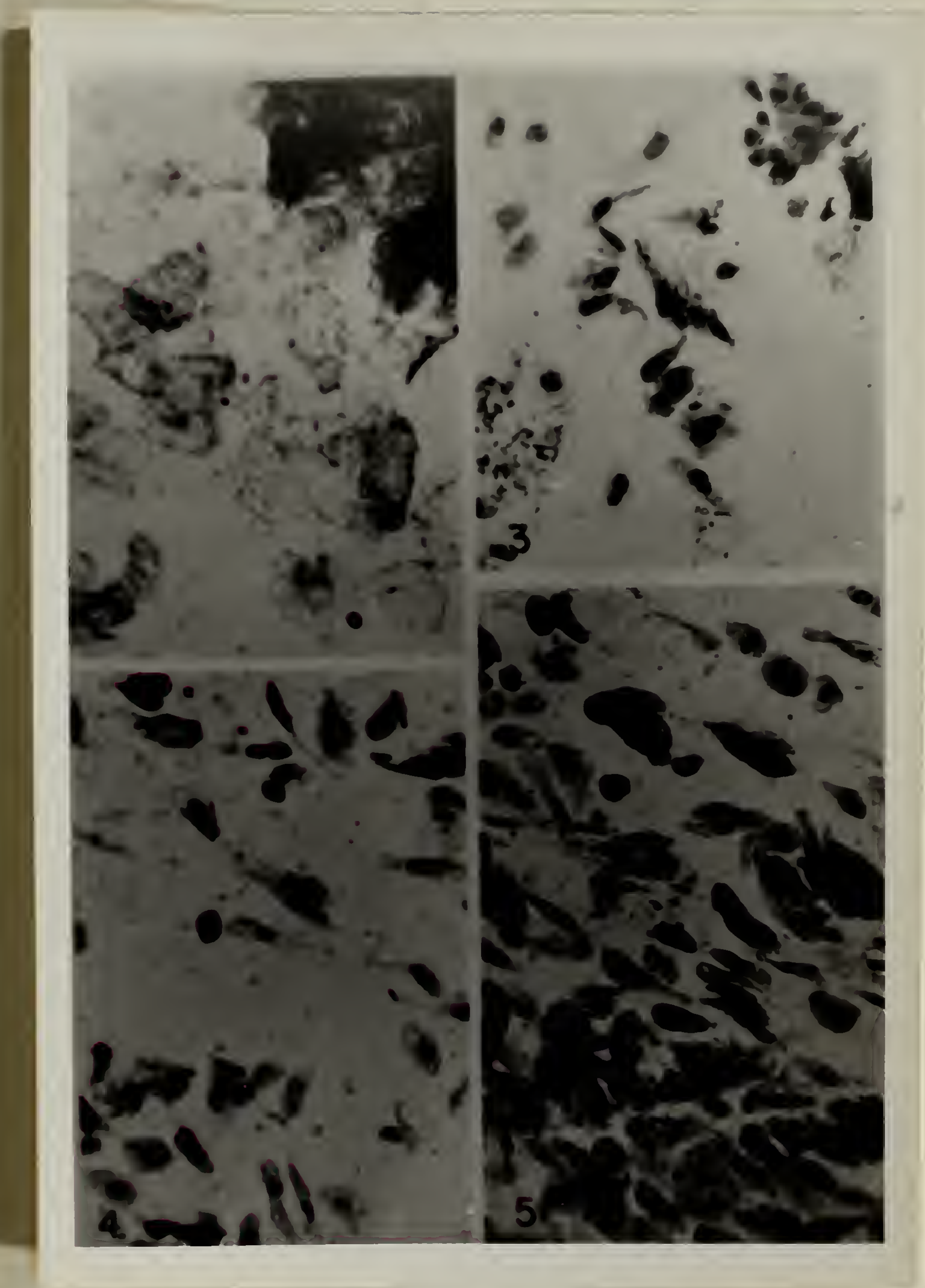
Ward's description of Metestrum B, which corresponds with the diestrum of the other authors, concurs with that given by Peczenick (1942) and Kent and Smith (1945). The latter investigators, however, confine the sparse cellular picture to the last twenty-four hours of diestrus, the preceding twelve hours being characterized by large numbers of leucocytes and cuboidal cells. In addition, they make no reference to the morphous debris mentioned by both Ward and Peczenick.

Kupperman, Greenblatt and Hair (1944), described the vaginal smear of diestrum as being characterized by large

numbers of leucocytes, and Sheehan and Bruner (1945) observed a large number of cells, predominantly nucleated epithelial cells, leucocytes, and a few large cornified cells, during diestrum, thus disagreeing with the above-mentioned description.

Figure 1 Proestrus Smear

Figure 2 Early Estrus Smear



Estrus Smear Figure 3

Late Estrus Smear Figure 4

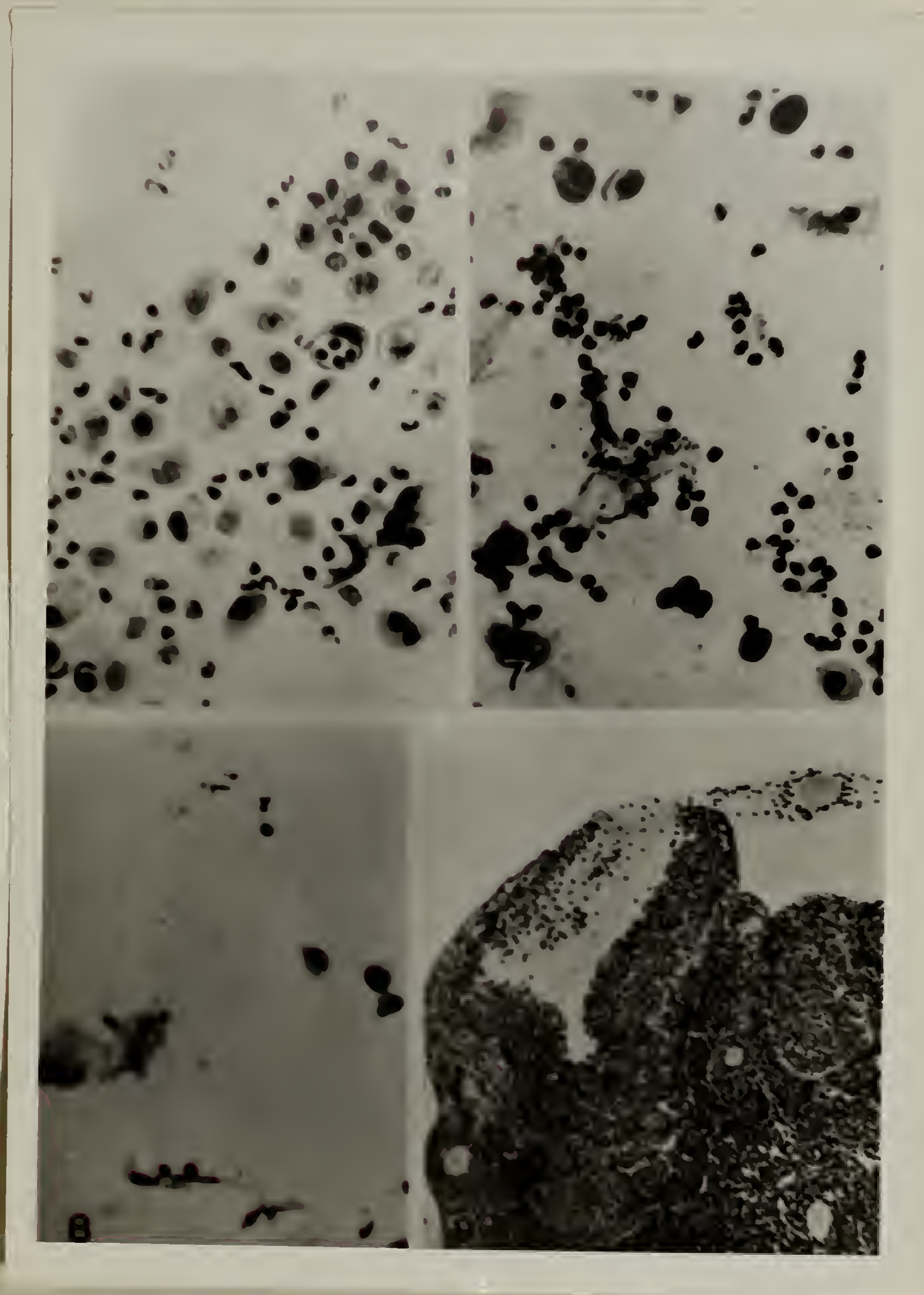
(Magnification x320; from Ward, 1946)

Figure 5

Metestrus A

Figure 6

Late Metestrus A



Metestrus B

Figure 7

Section of Ovary At Time of Ovulation

Figure 8 (x60)

(Magnification of Fig. 5,6,7 x320; from Ward,1946)

Time of Ovulation

In order to determine the time of ovulation, Ward (1946) killed a series of eight females in the middle of estrus, from midnight to 1:15 A.M. of day two. The ovary of one female, killed at midnight, contained six unruptured follicles in the later stages of the first maturation division. The ovary of an other female killed at midnight contained one ruptured and six unruptured follicles. All the ova were in the first stages of the first maturation division.

The ovary of a female killed at 12:30 A.M. contained two ruptured follicles and five unruptured follicles. The ovary of a female killed at 12:45 A. M. contained one ruptured follicle, one follicle in the process of rupturing, and four unruptured follicles.

Three females were killed at 1:00 A.M. The ovary of the first contained two ruptured follciles, two follicles about to rupture, and two unruptured follciles; of the second, one ruptured follicle, two near ovulation, and three unruptured follicles; of the third, three follicles that had ruptured a short time previously, two more in the process of ovulation and two that had not yet ruptured. The last female of the series, killed at 1:15 A.M., possessed in one ovary, one ruptured and two unruptured follicles.

It is evident that ovulation does not occur in all the follicles at the same time. The interval between the ovu-

lation of the follicles may extend over a considerable length of time, for in some ovaries one follicle was found to have ruptured and to have undergone definite post-ovulatory changes before other follicles in the same ovary had advanced very far in preovulatory changes. From the varying conditions seen in this series, Ward suggests that ovulation may extend over a period of one to two hours, and that the peak of ovulatory activity occurs approximately at 1:00 A.M. of day two, when the vaginal smear is rich in cells and the vagina displays a flocculent moisture. These findings substantiate the observations reported by Graves (1945).

All the animals killed on the morning of day two showed about the same extent of development of the corpora lutea, and the ovaries were in a more advanced stage than that seen in the ovulating animal. This indicates that ovulation is completed in a relatively short time.

Discussion

As can be seen from the survey of the literature on the estrous cycle of the hamster, there are a great many differences among descriptions given by different investigators. The major difficulty seems to be the difference in the various terminologies employed. This is indeed regretable in a field of scientific inquiry, in which there must be a common language in order to advance our knowledge of that field.

Kent and Mixner (1945) describe metestrum as the

few hours when matings may prove fertile. If this is true, how can they possible call this stage metestrum? There are other confusing differences in terminology. Are the "squamous cells" of Kent and Mixner (1945), and Kent and Smith (1945), the "cornified cells" of Kupperman, Greenblatt and Hair (1944), and the "epithelial cells" of Peczenick (1942) and Ward (1946) identical? Since there is an absence of photographs or camera lucida drawings, a clear definition of terms is essential. Ward (1946), who decries the confusion in terminology, contributes to it by introducing a new term, metestrum B. The benefit accrued from this change of the commonly used "diestrum" to metestrum B is not perceived by the author of this thesis.

Kent and Smith (1945) describe histological structures similar to those described by Ward (1946), but interpretate differently the time relation in the cycle. The former investigators state that estrus lasts twenty-seven hours, while Ward states that it lasts fourteen hours. They designate as metestrum that stage which Ward interprets as middle and late estrus, because she obtained fertile matings at this time and because ovulation occured during this interval. Since Kent and Mixner do not include the late estrous period in their estrous stage, it must be assumed that they have included in the estrous stage that which Ward describes as proestrum as well as a part of metestrum B.

Inasmuch as Ward obtained fertile matings as early as 6:00 P.M. of day one and as late as 9:30 A.M. of day two,

the intervening time must be considered the estrous period. The persistence of the estrous period into the morning characterized by mucous discharge from the vagina is a new finding. It is understood, of course, that there are individual variations in the timing of the cycle within the species pattern.

The fact that Ward found unusually small numbers of embryos in animals mated at 9:00 A.M. or after, and killed after the sixth or seventh day of pregnancy, may indicate that this elapsed time from ovulation, is approaching the life span of the egg. If the average ovulation time is at 1:00 A.M. of day two and matings may be fertile until at least 9:00 A.M., the life span of the egg must be at least eight hours, to which the time it takes the sperm to reach the egg must be added.

I feel that Ward's observations were the most accurate because she studied the cycles for the longest period of time, namely three years. In addition, she took smears at very frequent intervals, of twelve hours at the most and oftentimes every hour. The length of time which she assigns to the estrous period is corroborated by her mating experiments. The time relation of the various stages of the cycle were worked out by vaginal smears and also by studying serial sections of the ovaries, tubes and uteri from animals killed during each part of the cycle.

Perhaps most of her findings would be confirmed by the work of the other investigators in the field, if a common terminology could be worked out.

ACTION OF SEX HORMONES ON THE ESTROUS CYCLE

In the absence of any confirmatory work, I herein present the results obtained by Peczenick (1942) quite completely. He regularly observed the vaginal smears and the shape of the ovaries and uteri of his stock of female hamsters, distinguishing four groups:

Group	I	II	III	IV
No.	27	10	29	10
Age(months)		5-15	7-21	$3\frac{1}{2}$-23
Cycles	regular 4 day	irregular 2-6 day	regular 4 day	---
No. Fertile	27	4(younger than 10 months)	0	0
No. Sterile	0	2(6 months old) 4($10\frac{1}{2}$-16 months old)	29	10

Ten animals were given single injections of one, two, or forty micrograms of stilboestrol, and five animals were given injections of one or two micrograms of estradiol benzoate. All the animals belonged to groups I and III aged up to fourteen months. In two hamsters, one injected with one microgram of stilboestrol and the other with forty micrograms, the estrous cycle was unaffected. Estrous smears appeared in all the other individuals within forty to forty-eight hours after the injection. Those injected with doses of one or two micrograms of either substance exhibited characteristic estrous smears for less than twenty-four hours. While in the hamsters injected with forty micrograms of stilboestrol, these smears

persisted for two to five days.

Three females from group III, aged ten to fourteen months, were mated, two to four weeks after injection of forty micrograms of stilboestrol, with the same males that had failed to fertilize them before injection. Two of them produced litters while the third remained sterile.

Peczenick then injected in a single dose, four females of group IV, aged three-and-a-half to twenty-two months, with forty micrograms of stilboestrol. Estrous smears were provoked, which began, on an average, forty hours after the injection and persisted for three days. In the animals aged eleven and ninteen months, regular cycles were established seven and nine days, respectively, after the end of the period of provoked estrus. Eighteen days after the injection the animals were successfully mated, but the litters died within two to four days. The effect concluded with three regular four day cycles in the female aged twenty-two months, while in the one aged three-and-a-half months a spontaneous estrus occurred eleven days after the expiration of the reaction, but did not recur.

Large Doses

Three groups, each of five animals, (ten from group III and five virgin females) were injected daily with two hundred micrograms of stilboestrol, estrone, and estradiol benzoate, respectively, for six consecutive days. Peczenick

found that the three estrogens produced, in all the animals, a characteristic alternation of periods of estrous smears with leucocyte smears. The epithelial periods persisted for one to seven days after estradiol benzoate, one to ten days after stilboestrol, and two to six days after estrone. The leucocyte period lasted up to four days. Reaction ot the estrogens began, on an average, forty hours after the first injection and lasted, in animals injected with estradiol benzoate for twenty-five to twenty-six days, in those injected with stilboestrol, nine to ninteen days, and with estrone ten to twelve days.

Females, ovarectomized either before or after puberty, and injected with two hundred micrograms of estradiol benzoate or estrone for six consecutive days, exhibited different smears from those of normal females receiving the same dose. After forty hours epithelial smears appeared; subsequently, the epithelial cells disappeared almost completely, and the smears contained mainly slightly acidophil, basophil or pale cornified scales. These cornified smears alternated at irregular intervals with leucocyte smears. Shortly before the expiration of the reaction the number of cornified scales decreased and the number of epithelial cells again increased. Spontaneous estrus occurred as early as three to eight days after the last provoked estrous smears,and were followed by regular four day cycles. The animals were mated eight to twenty-four days after the disappearance of the estrogenic effect, but they remained sterile.

Action of Gonadotrophic Extracts

Four milligrams of AP118B (mixed horse anterior pituitary extract) produced in twenty-four-day-old hamsters estrous smears, ripe follicles, and luteinization; but in those eighteen days old, estrous smears without ripe follicles. Eighty micrograms of chorionic gonadotrophin produced estrous smears in twenty-four-day-old but not in eighteen-day-old females; while a dose of one hundred-sixty micrograms was effective in both groups. The estrous smears began ninety-six hours after injection and disappeared in less than twenty-four hours.

Five twenty-four-day-old females received four milligrams of AP118B daily for twelve days; five more of the same age received thirteen-hundred-twenty micrograms of chorionic gonadotrophin daily. Ninety-six hours after the first injection, estrous smears appeared. After seven to ten days the estrous smears changed their character and then consisted of a thin, slimy fluid, and could no longer be fixed by Leishmann's method. Ehrlich's haematoxylin was then used as the stain after fixing in methyl alcohol. Very small round cells, cell debris and scattered nuclei were seen, as well as columnar epithelial cells with small nuclei.

The smears contained most of these cells nine to thirteen days after the first injection. After this, the secretion of the thin slimy fluid increased, while the columnar

cells gradually diminished in size and number and seemed to disintegrate more readily.

Effect in Adult Females

Five animals from groups I and III each received single injections of eight or twenty-four international units of chorionic gonadotrophin; another five from the same groups, daily injections of two milligrams of AP 118B on three successive days. Estrus occurred, on the average, sixty-four hours after the injection of the chorionic gonadotrophin, or after the first injection of the Ap118B, and lasted four days independently of the stage of the estrous cycle. In the animals treated with the former, the regular estrous cycle was resumed four to eight days after the provoked estrus had ceased; in those treated with the latter, the cycle was resumed as early as four to five days after the last injection.

One group of five adult animals received injections of two hundred international units of chorionic gonadotrophin on five consecutive days, and another similar group four milligrams of AP118B on ten consecutive days. As early as three to five days later, shortly after the onset of provoked estrus, columnar-cell smears were obtained. Columnar cells were especially numberous and well formed in the earliest smears, subsequently decreasing rapidly in number, size, and state of preservation with the increase of mucous secretion.

Among them were seen isolated, larger, plate-like epithelial cells, also small cells of the estrous epithelial type.

In five more females, which had been injected daily with five milligrams of OMAO (old mare anterior pituitary extract) on ten consecutive days, the columnar cells first appeared four to seven days after the first injection. The estrous epithelium did not seem to decrease regularly, but varied irregularly or showed an actual increase after a short period of decrease.

Relationship of the Uterus and Ovaries in Pregnancy

The golden hamster is a species particularly favorable for contributing evidence on the action of the uterus on the ovary during pregnancy (Klein, 1938b). Klein made the observation that the removal of the entire uterus of the pregnant female causes the rapid and premature involution of the corpus luteum, resembling the reappearance of the estrous cycle. In addition, he observed that the ablation of only the embryos affects neither the corpora lutea, nor on the estrous cycle, provided that the placenta remains inserted into the uterine wall.

Action of Sex Hormones in Pregnancy

When female hamsters were ovariectomized during pregnancey the characteristic mucification of the vagina rapidly disappeared (Klein, 1938a). The injection of e strogen or

of progesterone alone ailed to produce mucification of the vagina of ovariectomized animals. However, when both estrogen and progesterone were injected simultaneously the vaginal mucification was maintained. It is, therefore, apparent that the vaginal mucification is caused by the simultaneous action of the two ovarian hormones.

Discussion

Peczenick's results (1942b)show certain peculiar results in which the golden hamster, in its reactions to sex hormones differs not only from other species but from other rodents. There is a marked difference between the effects of the same doses of estrogen on normal and ovariectomized animals. In the former, the estrogen produces a vaginal smear substantially the same as that shown in physiological estrus; it resembles the vaginal smears given by rats and mice, both in pro-estrus, and, exceptionally, in metestrus, and by ferrets (Hamilton and Gould, 1940) at the beginning of estrus. In ovariectomized animals, the estrus smears typical of hamsters were found by Peczenick only at the beginning and near the end of the reaction; the smears produced at the height of the reaction were substantially the same as estrous smears in rats and mice.

After the prolonged application of the gonadotrophic extracts a vaginal smear was produced which was different from those exhibited by other rodents, and which appears to be

a characteristic of the species. The fact that this vaginal smear was produced by progesterone and also found in pregnant animals show that it indicated the luteal phase of the vaginal mucosa. The columnar cells found in the smears are, in all probability, analogous to the mucifying cells found in the vaginal wall of mice and rats treated continuously with gonadotrophic extracts, and have been described as denoting "the second ovarian phase" (Marshall and Wiesner, 1932).

In the light of the discussion of the estrous cycle (see page 19), the question might arise as to whether Peczenick's description of estrus is accurate. Since it is in accord with Ward's finding (1946), I presume that Peczenick's histological description is correct.

The reaction of immature females to chorionic gonadotrophin demonstrate the endocrinological principle, namely, the reactivity of a tissue depends on the receptivity of the tissue and the amount of hormone involved.

THE ADRENALS OF THE GOLDEN HAMSTER

Peczenick (1944) described the appearance of the normal male and female adrenals in the golden hamster (Figs. 9 & 10). The author found a difference in the respective weights of the adrenals that is the reverse of the relationship observed in rats and mice (Bourne and Zuckerman, 1940); (Kupperman and Greenblatt, 1947), that of the male being greater than that of the female. It was found that the medullary diameters do not differ, but that the medullary total diameter ratios are different. This implies that the greater weight of the male adrenal is attributable at least in part, to the greater size of the male cortex. The weight of the male adrenal was found to be about .02% of the total body weight and the female adrenal approximately .008% of the total body weight. This dimorphic difference begins to appear with the beginning of androgenic activity in the male, as evidenced by the appearance of pigment spots on the dorso-lateral surface of the skin, about one third the distance between the xyphoid process and the iliac crest (Kupperman and Greenblatt, 1947).

In observing the external size of the adrenals, it was found that the left adrenal is approximately one and one half times the size of the right in both the male and the female. This condition is similar to that found in rats and mice.

There has been no evidence presented that there is

[illegible]

[illegible]

[illegible]

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[illegible]

an X-zone in the cortex, as has been found in young mice.

A sex-difference in the vacuoloization of the cortex was also observed by Peczenick (1944). The number and distribution of these vacuoles varied with the season, and in the female, also with the age and with the stage of the estrous cycle. In animals observed before or during the breeding season the male adrenal exhibited a vacuolized zone in the outer layers of the fasciculata (see Fig. 9), whereas the cells of the broader flatter inner zone were more compact. In diestrous and estrous females the vacuoles were more widespread and extended through the entire fasciculata to the reticular zone. The vacuolization seemed to become slightly increased during estrous. During metestrous the cortex appeared thickened and the vacuolization was rather similar to that of the male adrenal.

In animals observed during October and November, the vacuolization of the cortex of both the male and the female was greatly reduced. In senile females examined in March, the reticularis and the inner layers of the fasciculata were more vacuolized than the outer layers.

A sex-difference in the vacuolization of the adrenal cortex is also found in the guinea-pig (Kolmer, 1912; Allen and Bern, 1942). In this animal sudanophilic substances are found in the vacuoles of the fasciculata, as in the case of the rat (Golla and Reiss, 1942). In the golden hamster, however, the majority of the larger vacuoles did not contain

"visible fatty substances"(Bolles Lee, 1937). In chromated fresh sections droplets were seen which, with rare exceptions, were not stained with Sudan III and in some cases not even soluble in alcohol. The distribution of the "visible fatty substances" did not correspond to that of the vacuoles.

Relation of Environmental Conditions to the Adrenal

In golden hamsters adapted to 25°C. there was a marked increase in the region of the medulla taking a chromaffin stain (Kayser, 1939). In animals adapted to 8°C. there was very little chromaffin reaction. Since there is a reflex secretion of adrenin in the case of chemical temperature regulation, Kayser concluded that there was a definite relationship between the adaptation to temperature and chemical regulation. This indicates that the amount of adrenin present in the medulla is scarce in the adaptation to cold.

Peczenick (1944), following the histological data from the rat (MacKay and MacKay, 1926), namely, that after unilateral adrenalectomy the hypertrophied cortex of the remaining adrenal increases in activity, studied the reaction of the vacuoles in the hamster adrenal cortex under experimental conditions that would alter the activity of the cortex.

In agreement with the findings of the effect of extreme environmental temperatures on the activity of the cortex of the rat (Flexner and Grollman, 1939), Peczenick found that when the temperature fell suddenly vacuoles were practically

absent from the cortex. Lipoid material was confined to tiny droplets in the reticularis and the adjacent layers of the fisciculata.

Upon exposure to 35°C. for a period of four weeks, vacuoles were almost or completely absent from the fasciculata. Lipoid material was absent from the fasciculata save from the innermost layer, but the glomerulosa and the reticularis were crowded with sudanophilic lipoid droplets. In addition, the juxtamedullary layer of the reticularis exhibited peculiar small cells surrounded by dilated capillaries.

Effect of Sex Hormones on the Adrenals

In castrated male hamsters, the adrenal cortex reacted in the same way as in males kept at a high temperature. The ratio of the adrenal weight to the total body weight also kept decreasing (Peczenick, 1944). Kupperman and Greenblatt (1947) confirmed this finding with their description of the dimorphism of the adrenals due to androgenic activity. The histological picture of the cortex of the ovariectomized female resembled that of the normal male, save that the vacuoles were small, the large vacuoles being entirely absent. Peczenick gave neither the absolute weight of the adrenal nor any quantitative measurements as to its size.

In contrast to Kupperman and Greenblatt, who claimed no specific effect of sex steroids on the adrenals; Peczenick (1944) found that vacuolization of the cortex of the male cas-

trate was almost completely restored to normal upon treatment with androgens. However, the reticularis retained the characteristics of castration, many sudanophilic elements and small cells surrounded by dilated capillaries.

In ovariectomized females the administration of testosterone propionate caused a further reduction of vacuolization of the cortex, or a complete absence of vacuoles. In those females ovariectomized before maturation a normal male histological picture of the cortex was found.

The administration of estrogens to castrated males produced no change, except that in two cases the vacuolization was increased and distributed as in the female type. The reticularis of the two males showed no change from the castrate condition.

The administration of estrogens provoked different effects in the female castrate and non-castrate. In the female non-castrate, the cortex was moderately hyperaemic and vacuolization was increased. In the castrates, an exaggerated hyperaemia was exhibited and the spaces filled with erythrocytes and blood pigments. The cells were solid, the columnar arrangment in the fasciculata was lost and there was no distinction between the fasciculata and the reticularis.

In aged females injected with anterior pituitary gonadotrophin, the vacuolization of the cortex was not increased. The cells were more compace and had almost no vacuoles. No increase in cell division was detectable by the

colchicine test. In those injected with colchicine alone there was an increase of vacuolization, showing that the alkaloid did not inhibit the vacuolization of the cortex.

Discussion

The question arises as to whether the described actions of the active substances on the cortex are hormone effects or symptoms of a non-specific alarm-reaction (Selye, 1936). It would seem that androgens have a specific hormonal effect on the adrenal, for castration of the male produces effects that can be decreased or prevented by replacement therapy.

The circumstances are more complicated in the case of the female. In ovariectomized females, the sex-difference in adrenal weight is equalized, but neither this effect nor the reaction observed histologically is at all neutralized by the administration of estrogens. The adrenals show in their response to estrogens a dependence on the ovaries similar to the vagina (Peczenick, 1942). It is possible that in non-castrates the large doses of estrogens may be so weakened in their action on the adrenal cortex that, even as a nociferous stimulus, they may lead to an increased activity of the cortex, but never to exhaustion. Therefore, an increase in the vacuolization in non-castrated females treated with estrogens does not prove the hormonal nature of the effect. On the other hand, the possibility of converting the type of vacuolization may indicate a

specific hormonal effect of the estrogen.

Another problem is raised by the work of Peczenick (1944), namely, what is the function of the vacuoles? His description of the distribution of the vacuoles, as a great many in the outer layers of the fasciculata, decreasing in number in the inner layers, and none in the glomerulosa, is similar to Deane and Greep's (1946) description of the distribution of sudanophilic lipoid in the cortex of the rat. However, Peczenick found little or no sudanophilic material in these vacuoles, but rather tiny droplets of lipoid distributed through the cortex, in the same manner as that of the rat (Deane and Greep, 1946).

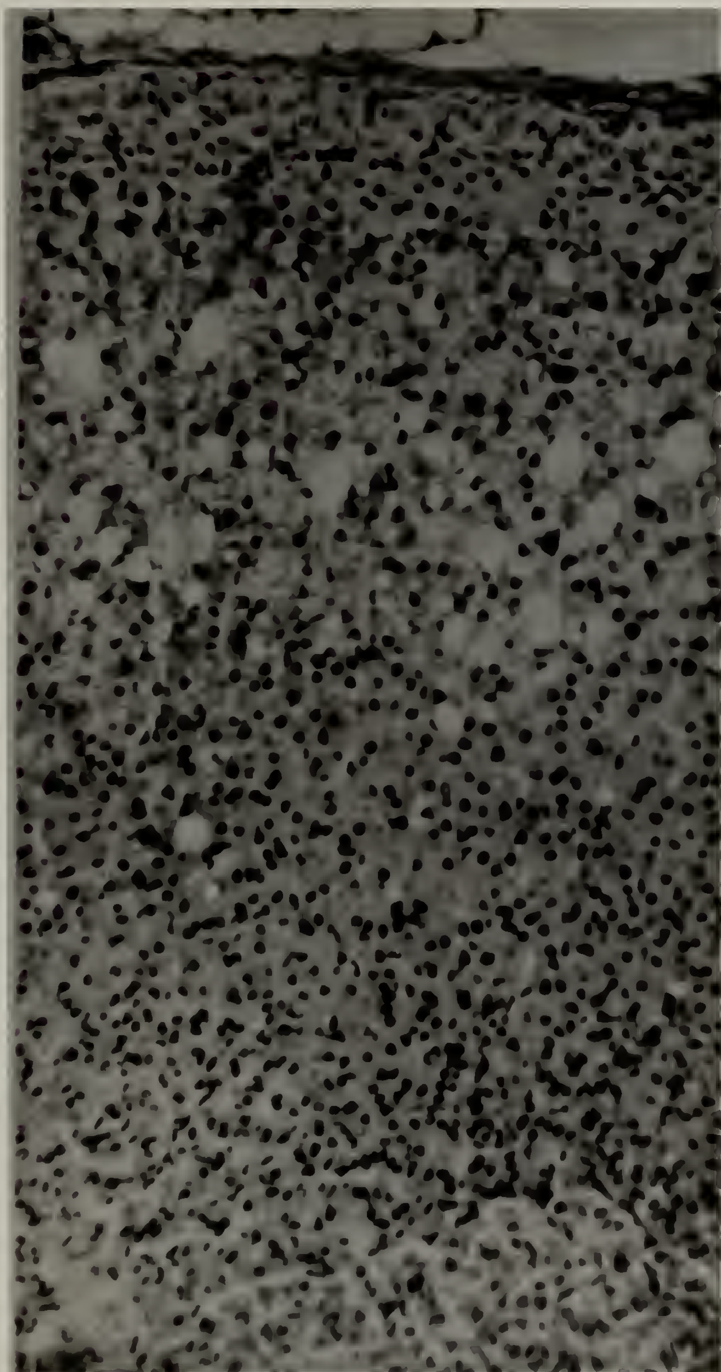
Peczenick fails to indicate what substance, if any, is secreted in the vacuoles. Yet, according to his results, the number and distribution of the vacuoles is an indication of the activity of the adrenal cortex. His statement, that in "cold animals" the vacuolization of the cortex is markedly reduced, seems to indicate that the greater the amount of vacuolization the greater is the activity of the adrenal.

Moreover, Peczenick (1944) observed that in "cold animals" the sudanophilic droplets were confined to tiny droplets in the reticularis and the adjacent layers of the fasciculata, which is in accord with the results obtained by Greep and Deane (1946). Therefore, it seems as if the vacuoles of the adrenal cortex of the hamster react in the same way as

does lipoid material. The vacuoles can not contain cholesterol as that substance is stained with Sudan III. Perhaps, the cortical hormone itself or one of its constituents, that is not sudanophilic, is stored there.

Assuming that Peczenick's technique for the staining of lipoids was accurate, further work should be done to determine exactly what is in the vacuoles of the adrenal cortex of the hamster.

MALE TYPE OF ADRENAL VACUOLIZATION



Cortex

Medulla

Figure 9

(Magnification about x440; from Peczenick, 1944)

FEMALE TYPE OF ADRENAL VACUOLIZATION

Figure 10

(Magnification about x440; from Peczenick, 1944)

BILATERAL ADRENALECTOMY OF THE HAMSTER

Introduction

There are several reasons why a series of bilateral adrenalectomies were undertaken in the golden hamster. First, the hope that this investigation would add to our present limited knowledge of the endocrinology of the species. Second, since the hamster is especially suited for laboratory investigation, a successful operative technique would offer a convenient method of studying the functions and reactions of the mammalian adrenals. As an example, the hamster would be a convenient laboratory animal for the study of the relationship between the adrenals and growth processes, because the animal reaches maturity in such a short time. Third, the investigation has value as a necessary preliminary to the study of transplantation of the adrenals. The study of the degeneration and regeneration of the adrenal tissue would be of particular value at the present time, in order to provide further evidence for or against Deane and Greep's (1946) suggestion that Zwemer's "Escalator Theory" of the growth of adrenal cortical cells is inaccurate.

Peczenick, 1944, performed unilateral adrenalectomies on the hamster, in the course of his investigation into the action of sex hormones on the adrenal cortex; however, he did not describe his operative technique.

An operative technique for adrenalectomy of the hamster will be presented, in the body of this paper. A preliminary report on the physiological effects of bilateral adrenalectomy in the golden hamster will also be presented.

Method

Operative Technique

The first technioue attempted was a dorsal approach used by Dr. Wyman and Dr. tum Suden at the Boston University School of Medicine and by others in the rat. This technioue consisted of puncturing, with sharp forceps, the dorsal muscle wall over the site of the adrenal; spreading the muscle fibers with the forceps, and removing the adrenal. This did not prove feasible because of the extreme delicacy of the hamster's addominal wall and the position of the liver. It was found that the puncture could not be controlled well enough to avoid lesion of the liver, resulting in a severe hemorrhage, which obscured the entire field.

A ventral approach also proved unusable for it entailed too much manipulation of the gastro-intestinal tract and too much exposure of the abdominal cavity, resulting in fatal shock. The technioue finally found successful is herein presented, step by step.

Ether was used as the anesthetic. A bell jar, covered with a glass plate, was utilized for the anesthetizing chamber, while a wire-gauze cone provided a continous ether-air mixture throughout the operation. In order to immobilize the animal and to provide a maneuverable tabel, a frog board and clips were employed. Although the instruments were sterilized and a tincture of iodine applied to the operative field,

an absolutely aseptic technique was not used. Figure 11 demonstrates the pre-operative set-up.

The hamster was placed in the ether chamber for a period of two to four minutes (Fig. 12). When the animal became limp and began to breathe very rapidly, it was removed from the chamber, placed on the frog board, and secured and the ether cone was placed over the nostrils (Fig.13). Care must be taken in anesthetizing the hamster, for it was found that the margin between anesthetization and death was very narrow.

The hair on the operative field, which extends from the first lumbar vertebra to two-thirds the distance to the sacrum, was then clipped and a tincture of iodine applied to the area. A skin incision of three-and-a-half to four centimeters was made; and the skin was then freed from the abdominal muscles by separating the subcutaneous fascia (Fig. 14).

The field then exposed is as follows (Fig. 14): the anterior border of the field is at the level of the first lumbar vertebra, while the posterior border is approximately one-half the distance to the sacrum. Exposed by the incision are the Musculus lastissimus dorsi, the Musculus obliquus abdominis externus, and the anterior end of the lumbodorsal fascia. Landmarks are subcutaneous blood vessels, in the region of the angle of the eleventh rib and the vertebral column, and abdominal fat extending posteriorly from the angle out of the field (~~Drawing 1~~).
Figure

An incision was then made five to ten millimeters

from the apex of the angle, immediately above the abdominal fat, and between the blood vessels. This was done by snipping the muscle wall with sharp scissors. Care must be taken not to injure the intestinal tract, which often lies close to the area of incision. The incision is then expanded by extension of a pair of forceps, in a direction which is at right angles to the direction of the muscle fibers (Fig. 15). The incision was then held open by moderately curved forceps, exposing the kidney, the upper pole of which is overlapped by the liver (Fig. 15). The position of the adrenal in relation to the kidney is shown in Figure 16.

In order to bring the right adrenal into the field, curved forceps were placed under the upper pole of the kidney, which was then rotated upward (Fig. 17). The adrenal was surrounded by a small ring of fatty tissue and adhered closely to the kidney. Extreme care had to be used in removing the adrenal. The adrenal peduncle was clamped with curved forceps. Then the forceps holding the incision open were release, but allowed to remain in the incision, acting as retracters. With other forceps, the adrenal was worked free. Hemostasis took place quickly so that ligation was not necessary. The procedure for the removal of the left adrenal was the same, except that the adrenal can be seen without rolling the kidney.

The muscle incision may be left unsutured, unless over one centimeter in length. If sutures were employed, number 000 surgical silk was used (Fig. 18). Finally, the

skin incision was sutured with number 00 surgical silk. (Fig.19)

Experimental Procedure

Nine operated animals were placed on Rockland Mouse Diet and tap water. Eight operated animals were placed on saline solution (0.5% sodium citrate and 0.5% sodium chloride and Rockland Mouse Diet. Five other animals were blank operated and placed on tap water and Rockland Mouse Kiet. Five unoperated animals were used as controls and were placed on tap water and Rockland Mouse Diet.

The body weights and food intake of all the animals were recorded daily. Autopsies were performed upon death of any of the animals.

Results

Operative Results

Bilateral adrenalectomies were attempted on twenty-one hamsters, with seventeen of these surviving. Therefore, of the deaths occurred in the first operative series, which consisted of nine animals; while only one animal died during the operation, in the second series, which consisted of twelve animals.

The cause of death, in all cases, was asphyxiation, due to blocking of nasal passages. It seemed that too long exposure to the anesthesia in the ether chamber caused excess formation of mucous. The animal breathed sharply and noisely immediately prior to the cessation of respiration, which stopped suddenly.

Perhaps, it would have been better to use urethane as the anesthesia. Dr. Fulton of the Boston University Biology Department successfully used urethane, in the case of the hamster, in the course of his work upon the circulation of the cheek pouch. However, one serious disadvantage in the use of urethane is that two people are needed to administer it. Therefore , if one is working alone, ether remains as the preferable anesthesia, with great care being used in its administration.

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Physiological Results

Animals Placed on Tap Water

Of the nine animals placed on tap water and Rockland Mouse Diet, three died between the fifth and eighth days after the operation. Operative injury to the right lobe of the liver and the right kidney appeared to have caused the death of the animal that died on the eighth post-operative day (Chart 1).

Autopsy of the animal that died on the sixth post-operative day revealed no apparent adrenal insufficiency. The left love of the liver was slightly congested, marking the site of a possible operative injury. The cause of death was undetermined (Chart 2).

Adrenal insufficiency did not appear to be the cause of death of the animal that died on the fifth post-operative day. Upon autopsy the outer muscle layers of the stomach and duodenum were seen to be ulcerated, with an actual perforation in the stomach wall (Chart 3).

The six remaining animals have maintained their body weight fairly well (Graph 1; charts 4, 5, 6, 7, 8, 9). All are living, at the present time, with the exception of one animal sacrificed on the twenty-seventh post-operative day, in order to check the thoroughness of the operation. There was no macroscopic evidence of adrenal tissue remaining. At this writing, two of the group have survived for thirty days, one for

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

twenty-four days, and two for nineteen days.

Animals Placed on Saline Solution

Two of the animals placed on saline solution died within five days after the operaion. There was no evidence of adrenal insufficiency, but the lungs were markedly congested upon autopsy. Therefore, it seems as if an after-effect of the anesthesia was the cause of death (Chart 10).

One of the animals of the group died on the fourteenth day after the operation. Post-mortem examination revealed that the body fat had disappeared, with no other apparent signs of adrenal insufficiency (Chart 11).

Another animal of the group died on the eighth post-operative day. There were no evident signs of adrenal insufficiency upon autopsy. However, the intestines were perforated in two places. This could account for the weight loss of the animal (Chart 12).

Three animals of the group are still alive at the fifteenth post-operative day. Their weight loss has been very slight, with their weight curve remaining fairly steady (Graph 2; charts 13, 14, 15). Saline solution seems to have no effect on body weight. The weight curves of animals placed on saline are similar to the weight curves of the animals placed on tap water.

Blank Operated Animals

All the blank operated animals survived, except one that died from an undetermined cause. Their body weight has decreased from three to ten grams, while the individual animal shows a fairly constant weight curve (Graph 3; chart 16).

Normal Controls

One of the normal controls died on the ninth day after the beginning of the experiment from an undetermined cause. The four remaining animals are still living. The body weight of all the animals has increased or remained the same since the inception of the experiment (Graph 4; chart 17). No correlation could be made between food intake and body weight of any of the animals of all the groups.

Discussion

It is felt by this writer that a practical operative procedure for bilateral adrenalectomy of the hamster has been presented. That a few animals were lost because of overdose of anesthesia or because of operative injury is to be expected, for the investigator was unfamiliar with the technique. With added experience it is probable that close to one hundred percent success will be achieved.

The physiological results observed are most interesting, for they are contrary to our previous knowledge of the

function of the adrenal cortex. The golden hamster is the only species, thus far studied by endocrinologists, able to withstand and survive bilateral adrenalectomy, without administration of cortical hormones or salts.

Before going any further, it must be emphasized that these are tentative and preliminary results. The adrenalectomized animals must be followed for a much longer postoperative period and many more animals must be tested before any validity can be attributed to the results.

Some adrenal cortical tissue might have been left after the operation and might have regenerated, enabling the animals to survive. However, the fact that six animals have survived and maintained their body weights at a fairly constant level, and that a check exploration failed to reveal any regenerated tissue seems to indicate there is some other mechanism of compensation for the loss of the adrenals.

The first possibility that presents itself is that of microscopic rests of adrenal cortical tissue within the peritoneal cavity, sufficient for the survival of the animal. This is the line of investigation upon which this investigator is now working. In addition, I intend to perform additional series of adrenalectomies to check my results.

There ought to be fairly large amounts of this microscopic tissue, if any, for the weight loss of the adrenalectomized animals seem to coincide with the weight loss of the blank operated animals. In order to further control the con-

ditions of the investigation, litter mates should be used for comparison. This was not done in the present investigation.

If no accessory adrenal cortical tissue is found, the number of possible compensating mechanisms is very large, requiring much further investigation.

The above discussion is based on the belief that the investigation, as far as it has gone, is accurate. I hope, that it has, at least, pointed out the problem that is raised by the results of the study of the effect of bilateral adrenalectomy of the hamster.

Summary

1. A practical procedure for bilateral adrenalectomy of the golden hamster is described.

2. The possibility that golden hamsters are able to survive bilateral adrenalectomy, without adminstration of cortical hormone or electrolytic salts, is presented.

SUGGESTED PROBLEMS FOR FURTHER RESEARCH

1. The relation between the hormonal effect and the age of the end organ. The short gestation period and the short time needed for maturity makes the hamster especially well suited for this investigation.

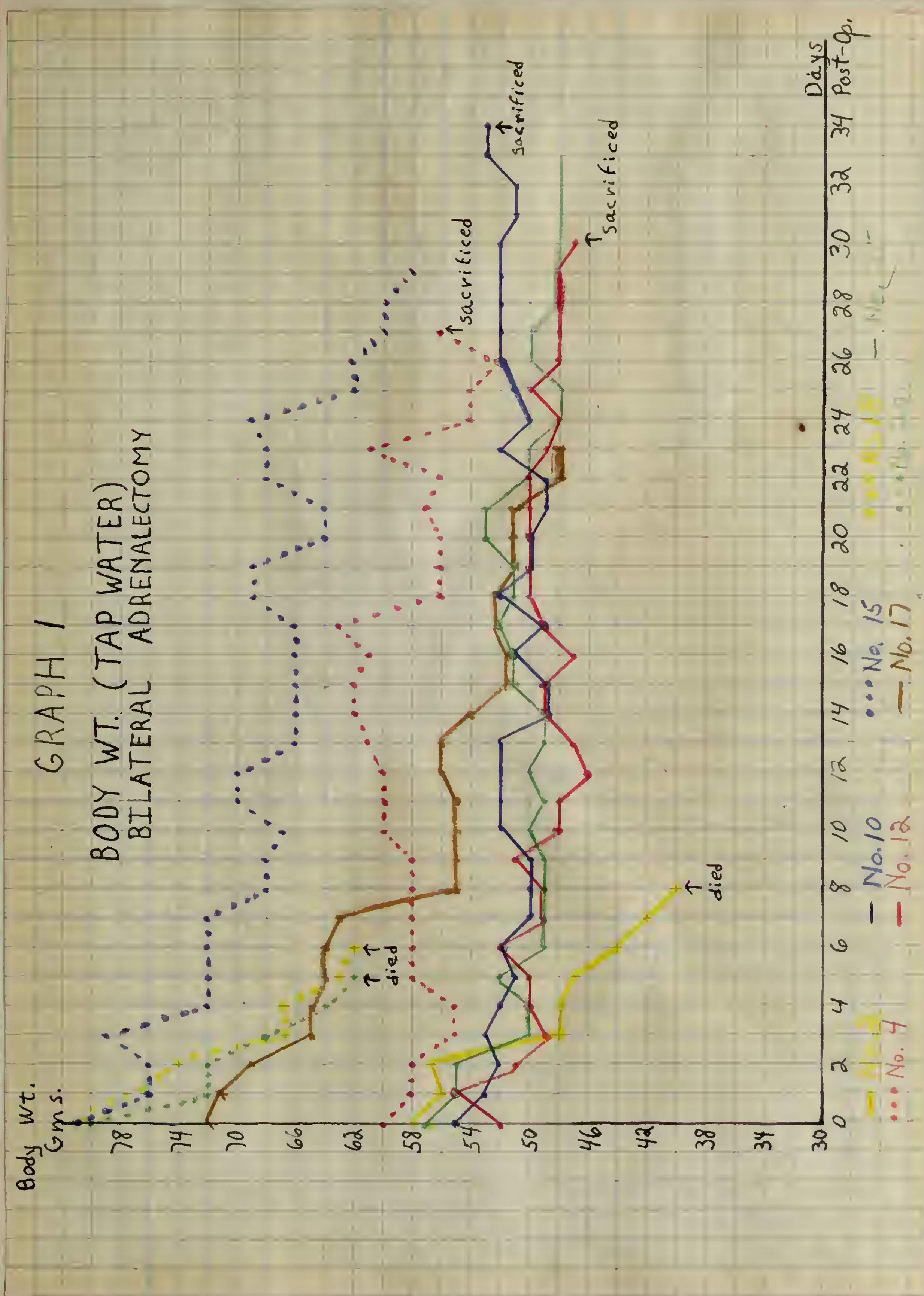
2. The function of Peczenick's vacuoles of the adrenal cortex.

3. The physiological effect of bilateral adrenalectomy in th hamster is now being investigated in the Boston University Biology Laboratories and offers many possibilities for research.

4. The morphology and histology of the pituitary, the thyroid, the pancreas, the testes, the ovaries, and uterus have not been adequately or accurately described, as yet.

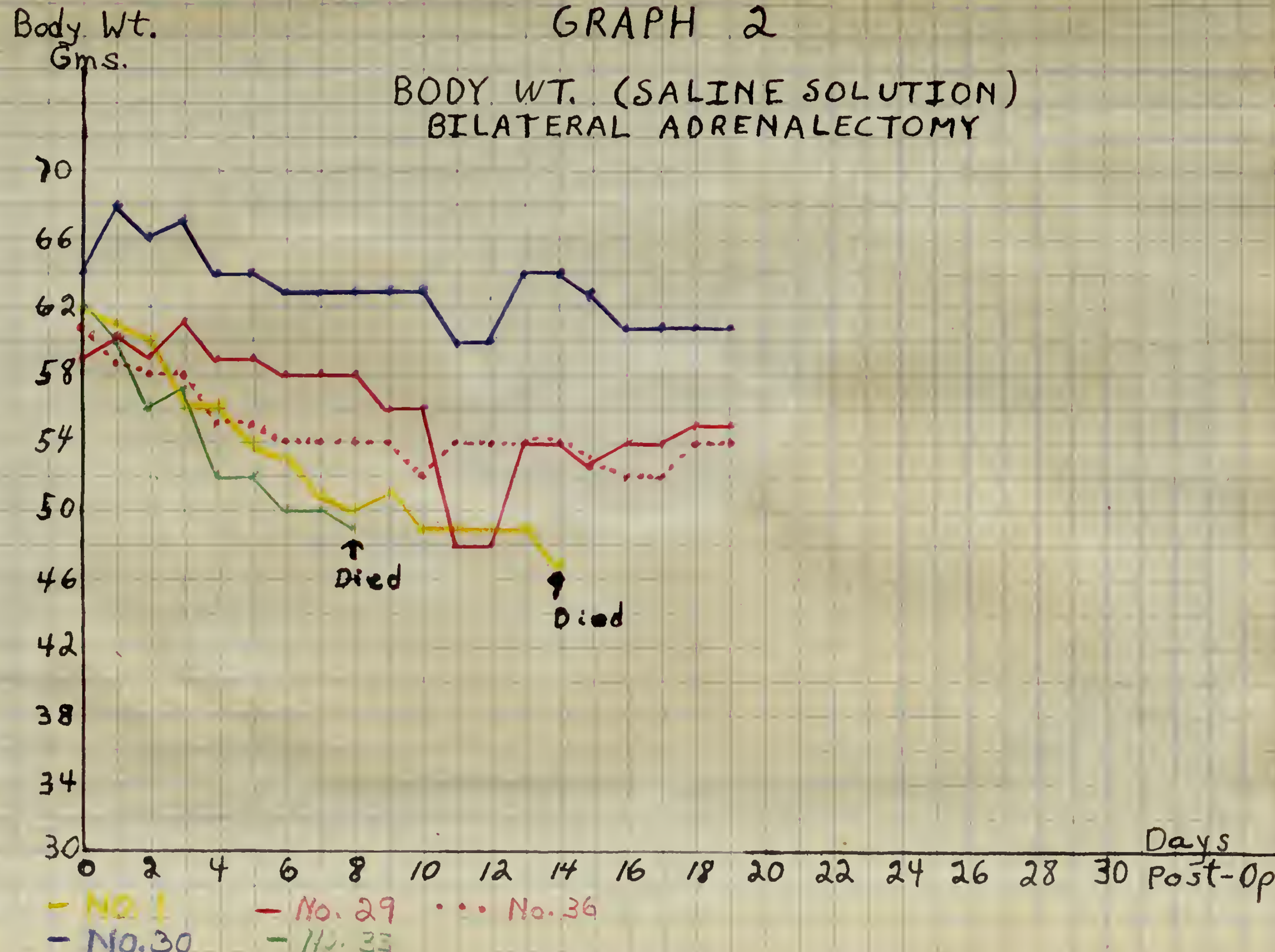
5. It would be of interest to make histological studies of the endocrine organs throughout the life span and also at different times of the year.

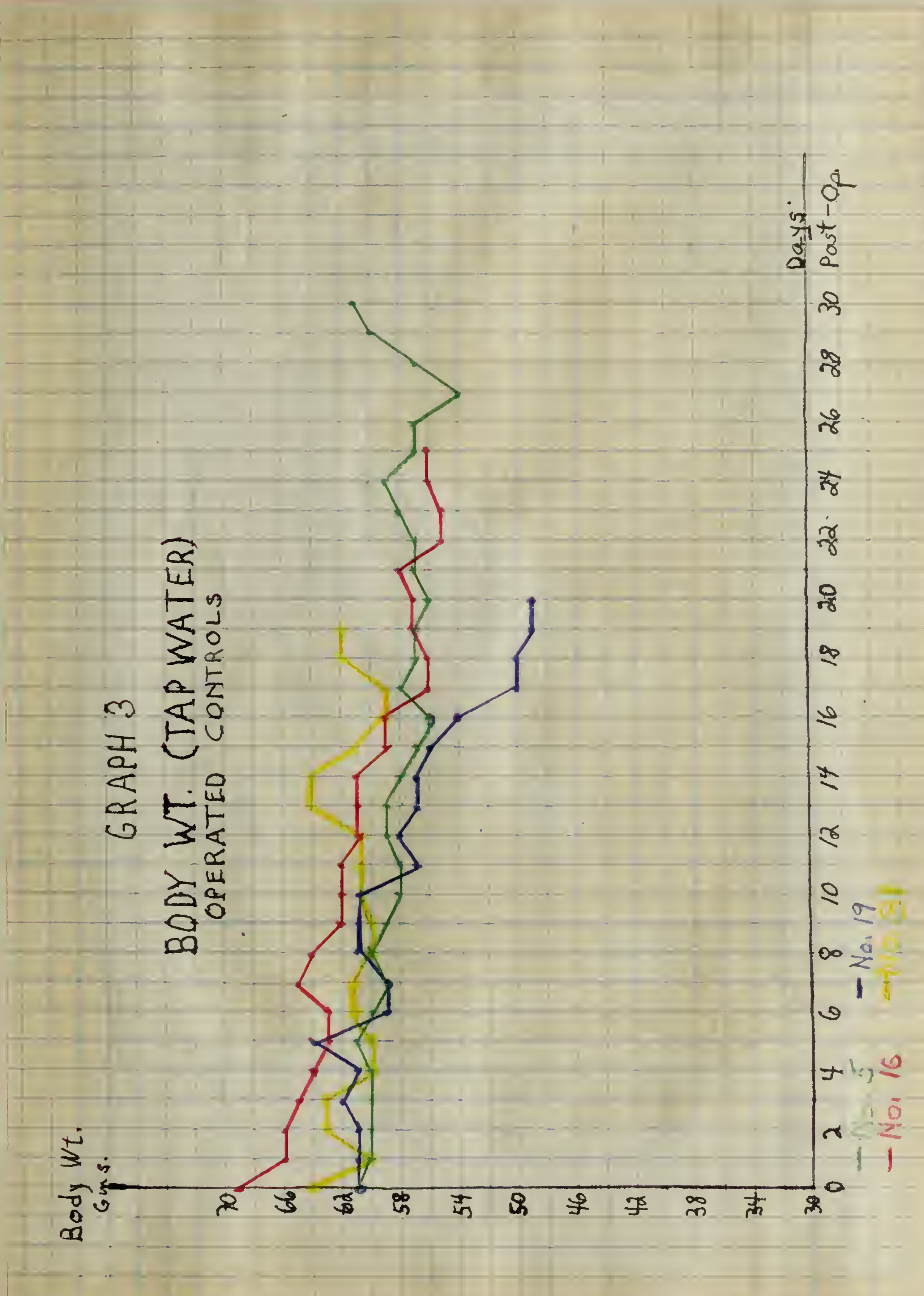
6. Since the hamster seems to differ in many ways from other species, it might be of some value to investigate the functions of all the endocrine organs.


GRAPH I
BODY WT. (TAP WATER)
BILATERAL ADRENALECTOMY
Body Wt. Gms.
Days Post-Op.
— No.10
— No.12
••• No.15
— No.17
••• No. 4
died
died
sacrificed
sacrificed
sacrificed

GRAPH 2

BODY WT. (SALINE SOLUTION)
BILATERAL ADRENALECTOMY




GRAPH 3
BODY WT. (TAP WATER)
OPERATED CONTROLS
Body Wt. Gms.
70
66
62
58
54
50
46
42
38
34
30
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Days Post-Op.
— No. 5
— No. 16
— No. 19

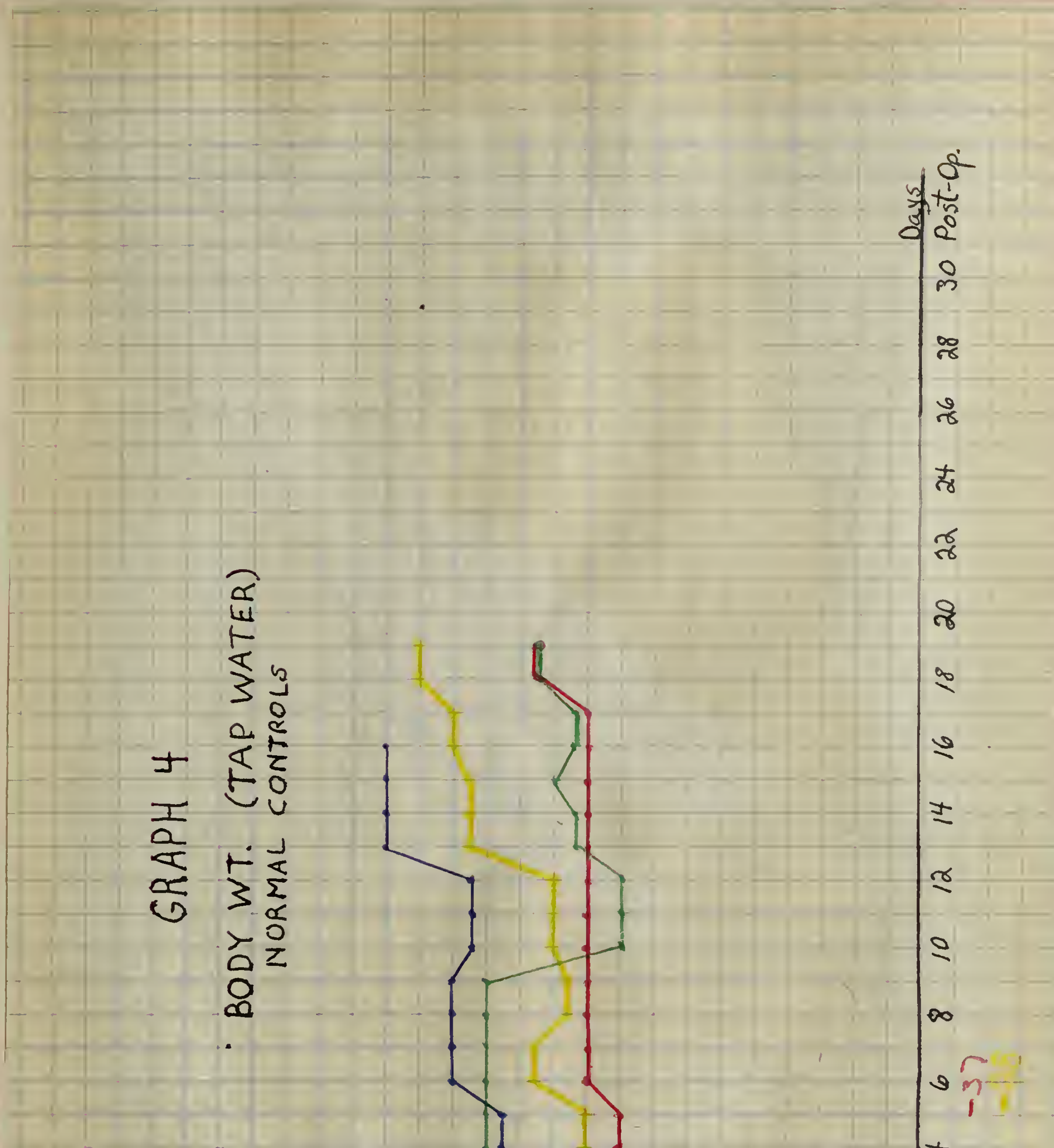

GRAPH 4
BODY WT. (TAP WATER)
NORMAL CONTROLS
Body Wt.
Gms.
70
66
62
58
54
50
46
42
38
34
30
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Days Post-Op.
-32 -35 -37 -40

Chart 1 Hamster No. 2

Tap Water Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Consumption (gm.)	Condition
0	58	---	20	---	Upper pole of right kidney injured in operation.Operative time 17 minutes.
1	56	17	17	3	Very active and alert. Sutures intact.
2	57	14	14	3	Good. Very active.
3	53	12	12	2	Good.
4	49	10	10	2	Alert. Getting weaker, however.Fur in good condition.
5	47	8	13	2	Weak,but still alert.
6	44	12	12	1	Fairly active and alert
7	42	10	10	2	Losing weight rapidly. Weak.
8	40	10	10	--	Found dead at 2:00 p.m Death must have occurred some hours earlier

Autopsy

Seminal vesicles and testes enlarged agreat deal. (Probably due to post-mortem change). Little or no body fat remaining. Blood vessels of intestines markedly distended. Liver spotty and congested. Spleen and thymus greatly enlarged. Lungs in good shape.

Chart 2

Hamster No. 18

Tap Water

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	81	--	12	-	Operative time 35 minutes. Hemorrhage in area of left kidney.
1	76	10	10	2	Active and alert.
2	74	6	6	4	Active and alert.
3	67	1	14	5	Active and alert.
4	67	14	14	0	Still active and alert.
5	63	13	13	1	Active and alert.
6	61	13			Found dead at 8:30 p.m.

Autopsy

Body fat normal. Lymph nodes not evident. Thymus not enlarged. A small area of congestion around the upper pole of the left kidney. The kidney itself was not injured. The left lobe of the liver appeared congested.

Chart 3

Hamster No. 20

Tap Water

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	82	--	12	-	Operative time 25 min.
1	74	12	12	0	Very active and alert.
2	73	11	11	1	Active and alert.
3	70	8	8	3	Active and alert. Incision healing well.
4	66	8	8	0	Rather weak. Walks shakily. Irregular muscle twitches.
5	64	8			Found dead at 5:00 p.m.

Autopsy

Not dead for long as auricular heart beat still present. Thymus normal. Lymph nodes not evident. Body fat reduced to some extent, but still present. Site of adrenals free from congestion or injury. Muscle incision almost completely healed.

The outer muscle walls of the stomach and duodenum appeared ulcerated. There also appeared to be an actual perforation of the stomach wall.

Chart 4

Hamster No. 4

Tap Water

Bilateral Adrenalectomy

Days Post-Op.	Wgtd (gm.)	Food Left (gm.)	Food Added (gm.)	Food Consumption (gm.)	Condition
0	60	--	22	---	10 minutes operative time. Upper pole of right kidney may have been injured in operation.
1	58	19	19	3	Good. Very active and alert.
2	58	15	15	4	Good.
3	57	10	10	5	Good.
4	55	5	25	5	Good.
5	59	24	24	1	Alert. Fur in good condition.
6	59	19	19	5	Good. Sutures still intact.
7	59	13	16	6	Good.
8	59	10	10	6	Good.
9	59	5	18	5	Good.
10	60	12	12	6	Good.
11	60	7	16	5	Good.
12	60	10	10	6	Good.
13	61	6	14	4	Good.
14	62	8	17	6	Good.
15	62	11	11	6	Good.
16	61	5	15	6	Good.
17	63	9	9	6	Good.

(cont.) Hamster No. 4

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Consumption (gm.)	Condition
18	60	6	6	3	Good. Drop in body weight might be due to lack of heat in the animal room.
19	56	-	18	6	Good. Heat still off in animal room.
20	56	12	12	6	Good.
21	57	7	16	5	Good.
22	57	10	16	6	Good.
23	56	12	12	4	Good.
24	61	6	10	6	Good.
25	54	4	14	6	Good.Sudden drop in body weight unexplain-ed.
26	54	8	13	6	Good.
27	52	12	12	1	Good.
28	56	10	10	2	Good.

Hamster sacrificed on the twenty-eigth day after the operation in order to check the operative technique. The animal was killed by an over-exposure to ether.

Autopsy

No evidence of adrenal tissue at poles of kidney--entirely removed by adrenalectomy. Body fat decreased very slightly in amount, otherwise a normal post-mortem appearance.

Chart 5

Hamster No.10

Tap Water

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	55	---	19	---	Operative time 20 minutes.
1	53	13	13	6	Good. Very active and alert.
2	52	7	20	6	Very active and alert.
3	53	17	17	3	Active and alert. Steady in weight.
4	52	14	14	3	Very active and alert.
5	51	9	13	5	Good.
6	52	10	10	3	Good.
7	51	6	6	4	Good.
8	51	2	17	4	Good.
9	50	15	15	2	Good.
10	52	13	13	2	Good.
11	52	11	12	2	Good.
12	52	7	14	5	Good.
13	52	9	9	5	Good.
14	49	5	5	4	Good.
15	49	---	16	5	Good.
16	51	9	9	7	Good. Active and alert. Wound well healed.
17	49	3	12	6	Good. Very active.
18	52	10	10	2	Good.
19	51	5	5	5	Active and alert.

(cont.) Hamster No. 10

Days Post-Op.	Wgt (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
20	51	--	14	5	Active and alert. Incision healed.
21	49	9	9	5	Active and alert.
22	49	4	14	5	Active and alert.
23	52	9	9	5	Good.
24	51	5	13	4	Active and alert.
25	51	7	11	6	Active and alert.
26	52	7	7	4	Good.
27	52	2	14	5	Good.
28	52	9	9	5	Good.
29	52	4	11	5	Good.
30	52	6	12	5	Good.
31	51	8	8	4	Active and alert.
32	51	4	14	4	Active and alert.

Sacrificed

Adhesion of left kidney and spleen. Adhesion of liver and left kidney. No evidence of adrenal tissue,macroscopicly. Viscera fixed in Bouin's.

Chart 6

Hamster No. 12

Tap Water

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	52	---	13	---	Operative time 15 minutes
1	56	11	22	2	Good. Active and alert.
2	51	20	12	2	Good. Active and alert.
3	50	8	15	4	Good. Active and alert.
4	51	9	9	6	Good. Active and alert.
5	51	5	12	4	Good.
6	52	8	8	4	Good.
7	49	5	5	3	Good.
8	49	2	13	3	Good.
9	51	9	9	4	Good.
10	48	6	6	3	Fairly good.
11	48	4	14	2	Fairly good.
12	46	13	13	1	Losing weight rapidly. Still fairly active.
13	47	9	9	4	Active. Seems to be in good condition.
14	49	6	6	3	Good. Active.
15	49	3	12	3	Good. Active.
16	47	8	12	4	Good. Active and alert.
17	49	6	11	6	Good. Hair beginning to grow back.
18	50	9	9	2	Good.
19	50	6	6	3	Good.

(cont.)

Hamster No. 12

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
20	50	2	12	4	Good.
21	50	8	8	4	Good.
22	50	4	11	4	Good.
23	49	6	6	5	Good.
24	48	6	11	0	Weak. Body cold. walks very shakily.
25	50	7	7	4	Good. Much stronger. No shakes.
26	48	4	4	3	Good.
27	48	0	13	4	Good.
28	48	11	11	2	Good.
29	48	10	14	1	Good. Active.
30	47	10	10	4	Good.

Sacrificed

No macroscopic evidence of adrenal tissue. Entire viscera fixed in Bouin's. Body fat normal. Normal post mortem picture.

Chart 7

Hamster No. 15

Tap Water

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	80	----	18	---	Operative time 30 minutes.
1	76	16	16	2	Good. Incision shows no infection. Sutures intact. Very active and alert. Weight drop may be due to lack of water.
2	76	13	13	3	Good. Very active and alert.
3	79	9	12	4	Good.
4	72	10	10	2	Fairly good. Active
5	72	8	8	2	Fairly good. Active.
6	72	4	3	14	Good.
7	72	10	10	3	Good.
8	68	6	6	4	Good. Active and alert.
9	68	1	13	5	Good. Active and alert.
10	67	8	12	5	Good. Wound almost completely healed. Active and alert.
11	70	5	12	7	Good. Incision completely healed.
12	70	8	8	4	Good.
13	66	4	4	4	Active and alert.
14	66	0	16	4	Active and alert.
15	66	11	11	5	Active and alert.
16	66	6	9	5	Active and alert.
17	66	5	16	4	Good.

(cont.) Hamster No. 15

Days Post-Op.	Wgt. (gm.)	Food Left. (GM.)	Food Added (gm.)	Food Intake (gm.)	Condition
18	69	10	10	6	Active and alert.
19	69	5	10	5	Active and alert.
20	64	5	5	5	Good.
21	64	0	16	5	Good.
22	68	9	9	7	Good. Active and alert.
23	68	3	13	6	Good. Active and alert.
24	69	8	8	5	Good.
25	62	4	4	4	Good.
26	62	0	18	4	Good.

Chart 8

Hamster No. 17

Tap Water

BIlateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	72	---	12	---	Operative time 35 minutes.
1	71	9	9	3	Recovered from operation in good condition. Active and alert.
2	69	6	6	3	Good. Active and alert.
3	65	4	4	2	Good. Active and alert.
4	65	1	12	3	Good. Active and alert.
5	64	9	9	3	Good. Active and alert.
6	64	3	14	6	Very active and alert.
7	63	11	11	3	Active and alert.
8	57	8	8	3	Active and alert. Incision healing well.
9	57	6	13	2	Good.
10	57	8	8	5	Good.
11	57	4	14	4	Active and alert.
12	58	11	11	3	Active and alert.
13	58	6	13	5	Good.
14	54	13	13	0	Good.
15	51	8	8	5	Good.
16	51	4	13	4	Active and alert.
17	52	9	9	4	Active and alert.
18	52	5	13	4	Good.
19	51	7	11	6	Good.

(cont.)

Hamster No. 17

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
20	51	6	6	5	Active and alert.
21	51	1	14	5	Active and alert.
22	48	11	11	3	Good.
23	48	9	15	2	Good.

Chart 9

Hamster No. 25

Tap Water

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	57	--	13	-	Operative time twenty minutes.
1	55	11	11	2	Active and alert.
2	55	9	9	2	Active and alert.
3	51	7	13	2	No evidence of infection. Active and alert.
4	51	10	10	3	Good.
5	52	8	8	2	Very active and alert.
6	49	4	4	4	Active and alert.
7	49	0	12	4	Active and alert.
8	49	8	8	4	Active. Incision healing.
9	49	4	10	4	Good.
10	50	6	6	4	Good.
11	49	2	14	4	Active and alert. Suture broken, but incision healing well.
12	50	9	9	5	Good.
13	49	4	4	5	Good.
14	49	0	12	4	Good.
15	51	8	8	4	Good. Incision healed.
16	51	4	12	4	Good.
17	52	6	13	6	Active and alert.
18	51	7	7	6	Active and alert.

(cont.)

Hamster No. 25

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
19	51	1	17	6	Good.
20	53	9	16	8	Good.
21	53	16	16	0	Active and alert.

Chart 10

Hamster No. 34

Saline Solution

Bilateral
Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	62	---	18	---	Operative time 12 minutes. Left incision not sutured.
1	59	17	17	1	Good. Active and alert. Incision in good condition. Sutures not broken.
2	54	14	14	3	Good. Sutures ripped out.
3	52	11	17	3	Good. Active and alert.
4	50	16	16	1	Very,very weak.
5	50	16	16	1	Very, very weak.Dying. Body cold. Gasping, forced breathing. Died at 5:00 p.m.

Autopsy

Left muscle incision healed. Right muscle incision healed. Body fat not reduced.Blood clot in region of left adrenal. Right adrenal area clear. No sign of congestion. Lungs slightly congested. Thymus normal.

Chart 11

Hamster No. 1

Saline Solution — Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Consumption (gm.)	Condition
0	62	---	28	----	20 min. operative time Recovered in 15. min.
1	61	28	28	0	Very active.Sutures intact.
2	60	23	23	5	Active.No infection
3	58	20	20	3	Active and alert.
4	56	18	18	2	Active and alert.
5	54	17	17	1	Good. Loss in weight may be due to lack of drinking water.
6	53	16	16	1	Good.Fur in good condition.
7	51	13	13	3	Good.
8	50	10	10	3	Fairly active.
9	51	7	11	3	Good.
10	49	7	21	4	Good.Fairly active.
11	49	18	18	3	Less active.
12	49	17	17	1	Getting weaker.
13	49	14	14	3	Weak, not active.
14	47	13	13	1	Very weak,no activity at all.

Died on 14th day post-op.Period before death characterized by extreme lassitude and weakness. Periodic muscle twitches and occassional convulsive movements of the body.

Autopsy

Performed immediately after death. Body fat had disappeared.Lymph nodes not too evident. Thymus slightly enlarged. Blood vessels in mesentary extended to some degree. Lungs in good condition.

Chart 12

Hamster No. 33

Saline Solution

Bilateral Adrenalectomy

Days Post-Op.	wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	62	--	10	-	Operative time 20 minutes.
1	60	7	7	3	Good. Very active and alert.
2	56	3	16	4	Very active and alert.
3	57	14	14	2	Very active and alert.
4	52	12	12	2	Active and alert. Incision healing well.
5	52	11	11	1	Active and alert.
6	50	9	9	2	Very active and alert.
7	50	8	8	1	Active and alert.
8	49	8			Found dead at 12:30 p.m.

Autopsy

Body fat normal. Lymph nodes not evident. Thy mus normal. Lungs normal. Possible perforation of the intestine.

Chart 13

Hamster No. 29

Saline Solution

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	59	---	13	---	Operative time 15 minutes
1	60	10	10	3	Good. Very active. Sutures still in place.
2	59	5	14	5	Good. Sutures in place. Incision healing.
3	61	9	9	5	Good. Sutures in place. Incision healing.
4	59	6	6	3	Active and alert. Incision healing well.
5	59	2	17	4	Active and alert. Incision healing well.
6	58	4	4	3	Active and alert.
7	58	10	10	4	Active and alert.
8	58	6	6	4	Active and alert.
9	56	1	14	5	Active and alert.
10	56	9	9	5	Active and alert.
11	48	5	5	4	Good.
12	48	0	19	5	Active and alert.
13	54	16	16	3	Active and alert.
14	54	12	12	4	Good.
15	53	6	14	6	Good.
16	54	9	9	5	Good.
17	54	4	17	5	Active and alert.
18	55	13	13	4	Active and alert.

Chart 14

Hamster No. 30

Saline Solution

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	64	---	14	---	Operative time 15 minutes.
1	68	11	11	3	Extremely active.
2	66	5	10	6	Good. Sutures in place. Incision healing well.
3	67	8	8	2	Good. Incision closed.
4	64	4	4	4	Good. Incision healing well.
5	64	1	16	3	Active and alert.
6	63	12	12	4	Active and alert.
7	63	8	8	4	Active and alert.
8	63	4	8	4	Active and alert.
9	63	4	12	4	Active and alert.
10	63	6	10	6	Active and alert.
11	60	5	5	5	Active and alert.
12	60	0	18	5	Active and alert.
13	64	15	15	3	Active and alert.
14	64	12	12	3	Active and alert.
15	63	6	14	6	Active and alert.
16	61	8	8	6	Active and alert.
17	61	2	16	6	Active and alert.
18	61	12	12	4	Active and alert.
19	61	8	18	4	Active and alert.

Chart 15

Hamster No. 36

Saline Solution

Bilateral Adrenalectomy

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	61	---	13	---	Operative time 20 minutes.
1	59	12	12	1	Good. Active and alert.
2	58	7	11	5	Good. Incision healing well. Active and alert.
3	58	7	7	4	Active and alert.
4	55	4	4	3	Incision healing well.
5	55	1	14	3	Active and alert.
6	54	11	11	3	Active and alert.
7	54	7	9	4	Active and alert.
8	54	6	6	3	Active and alert.
9	54	1	14	5	Active and alert.
10	52	9	9	5	Active and alert.
11	54	5	5	4	Active and alert.
12	54	0	14	5	Active and alert.
13	54	10	10	4	Active and alert.
14	54	6	12	4	Active and alert.
15	53	6	10	6	Active and alert.
16	52	5	5	5	Active and alert.
17	52	0	15	5	Active and alert.
18	54	11	11	4	Active and alert.
19	54	6	17	5	Active and alert.

Chart 16

Hamster No. 5

Tap Water

Operated Control

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	61	--	21	--	Operative time 35 minutes.
1	60	14	14	7	Active. No sign of infection.
2	60	11	14	3	Good.
3	60	11	11	3	Good.
4	60	8	20	3	Good.
5	61	17	17	3	Good.
6	60	13	13	4	Good.
7	59	7	14	6	Good.
8	60	10	10	4	Good.
9	59	5	17	5	Good.
10	58	11	11	6	Good.
11	58	6	15	5	Good.
12	59	12	12	3	Good.
13	59	9	15	3	Good.
14	58	11	11	4	Good.
15	57	7	7	4	Good.
16	56	4	17	3	Good.
17	58	13	13	4	Good.
18	57	9	9	4	Good.
19	57	5	13	4	Good.

(Cont.) Hamster No. 5

Days Post-Op.	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
21	57	5	5	5	Good.
22	57	0	16	5	Good.
23	58	7	10	9	Good.
24	59	5	9	5	Good.
25	57	5	5	4	Good.
26	57	1	15	4	Good.
27	54	14	14	1	Good.
28	58	8	13	6	Good. Incision almost completely covered by new hair growth.
29	60	10	10	3	Good.
30	59	5	5	5	Good.

The animal is still alive, but the food intake and body weight determinations were discontinued thirty days after the operation.

Chart 17

Hamster No. 32

Tap Water — Normal Control

Days	Wgt. (gm.)	Food Left (gm.)	Food Added (gm.)	Food Intake (gm.)	Condition
0	50	--	10	--	Good.
1	51	9	9	1	Good.
2	55	3	12	6	Good.
3	56	11	11	1	Good.
4	55	8	8	3	Good.
5	55	4	15	4	Good.
6	58	9	9	6	Good.
7	58	4	10	5	Good.
8	58	0	17	10	Good.
9	57	12	12	5	Good.
10	57	7	7	5	Good.
11	57	1	15	6	Good.
12	62	11	11	4	Good.
13	62	8	14	3	Good.
14	62	8	14	6	Good.

Figure 11

Pre-operative Set-up

Figure 12

Ether Chamber

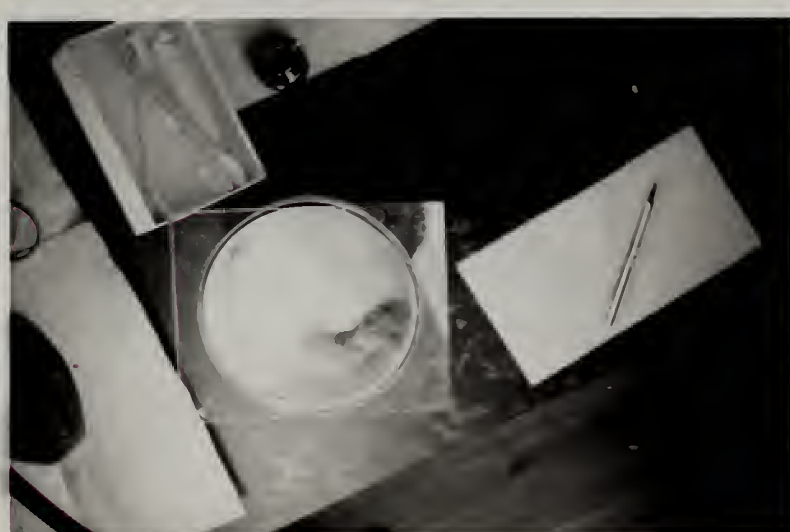


Figure 13

Hamster On Frog Board

Figure 14

Skin Incision

LANDMARKS FOR OPERATION

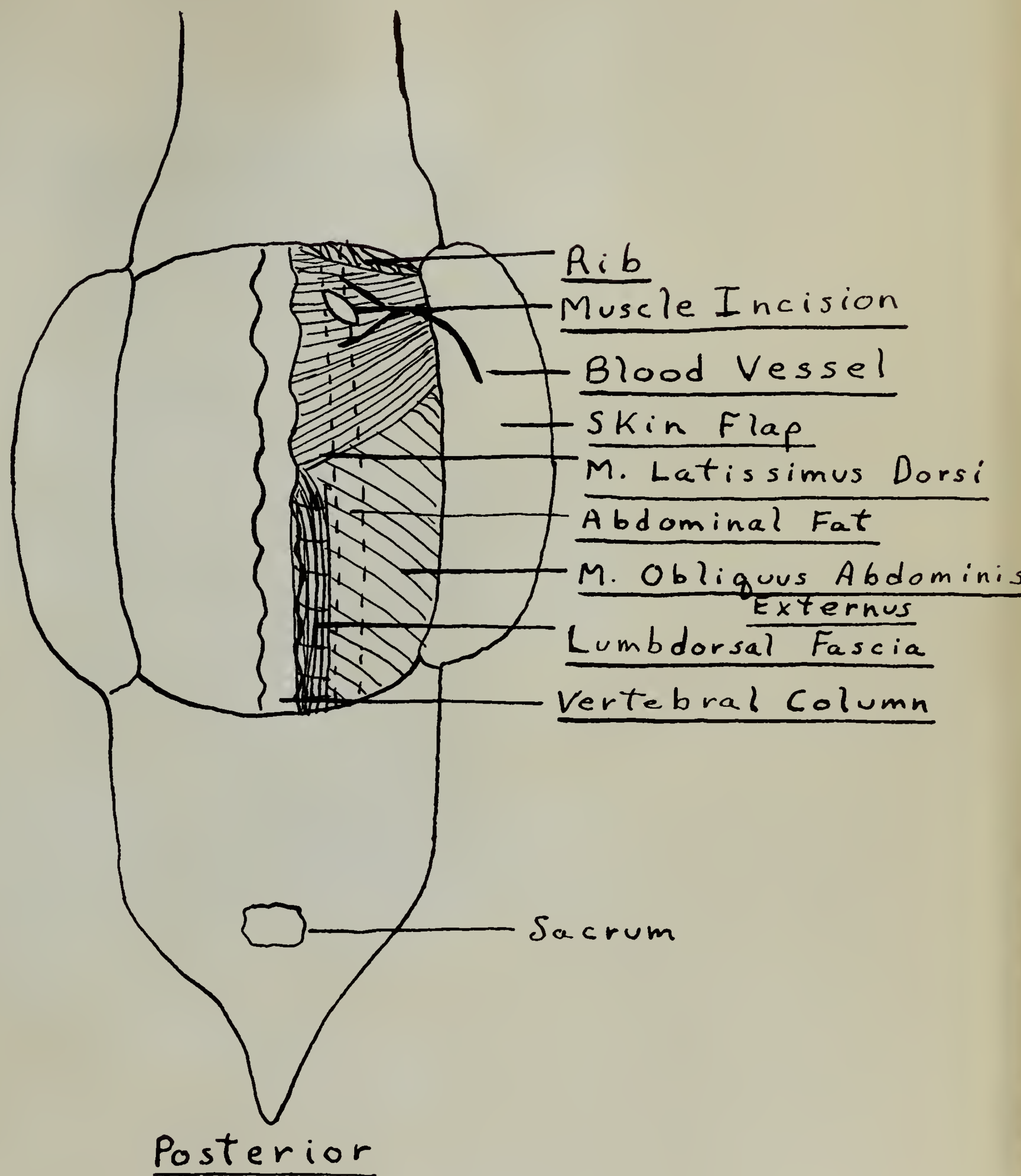


Figure 15

Muscle Incision

Figure 16

Cross Section Of Hamster At Level Of Third Lumbar Vertebra

Dorsal

Right

Left

Figure 17

Exposure Of Adrenal

Figure 18

Muscle Suture

Figure 19

Skin Suture

ABSTRACT

At least one species of hamster, Cricetus frumentaris, exhibits cyclic seasonal activity. The animals display a decline of general activity between March and September, and the reverse between September and March.

After birth the golden hamster, Cricetus auratus Waterhouse, does not continue the rapid growth of the prenatal period. Sexual maturity is reached at the age of thirty-six days, in both the male and female.

Gonadotrophins stimulate the growth of the male sex organs, the ovary, and the female accessory organs; the peak of reactivity being reached at sixteen days of age, thirty-six days of age, and six and ten days of age respectively.

Androgens and estrogens do not affect the growth of the ovary, but do affect the growth of male sex organs and the female accessory organs. The peaks of reactivity being the same as that for gonadotrophins.

It has been found that the estrous cycle of the hamster can be determined by vaginal smears. The cycle lasts for four days, proestrum lasting for approximately three hours, estrum approximately fifteen hours, metestrum approximately twenty-seven hours, and diestrum or metestrum B approximately fifty-one hours.

Estrous cycles may be provoked in anestrus females by injection of estrogens. There is a difference in the his-

tological picture of the estrous smear provoked in anestrus females, and that of the estrus smear provoked in ovariectomized females.

Gonadotrophins are effective in provoking estrus in immature animals as well as in adults.

A sex-difference in the histology of the adrenal cortex of the hamster is described. The conspicuous feature of the cortex is non-sudanophilic vacuoles. Temperature change in the environment results in a change of the histological appearance and activity of the cortex. It seems that androgens have a specific hormonal effect on the adrenal cortex; whereas, it is uncertain whether estrogens exert any hormonal effect on the adrenal cortex.

An operative technique for bilateral adrenalectomy of the hamster is described. The possibility that golden hamsters are able to survive bilateral adrenalectomy, without administration of cortical hormone or electrolytic salts is presented.

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